

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A method for identifying an MHC-binding peptide for an MHC monomer, or modified MHC monomer, said method comprising:

a) incubating under a suitable liquid phase conditions condition a sample a ternary complex comprising:

at least one ~~MHC~~ HLA-A2 monomer or modified ~~MHC~~ HLA-A2 monomer having bound thereto a template MHC-binding peptide and, ~~wherein the MHC monomer is HLA-A2, further comprising beta-2 microglobulin, and wherein the modified MHC~~ HLA-A2 monomer maintains the ability to assemble into a ternary complex with the template MHC-binding peptide and beta-2 microglobulin, and wherein said monomer is produced in an expression system selected from the group consisting of a prokaryotic system, a yeast system, a plant system, and an insect system;

an excess amount of a first competitor peptide, and

a tracer MHC-binding peptide tagged with a detectable label, wherein said tracer peptide competes with the first competitor peptide and the template peptide for binding to the monomer,

~~so as to allow competition between the first competitor peptide, the template peptide, and the tracer peptide for binding to the MHC monomer or modified MHC monomer,~~
wherein the template peptide has a lower or intermediate affinity ~~as compared with~~ than the tracer peptide for the monomer; and

b) determining a difference in signal produced by the detectable label in the sample as compared with signal produced solely by monomer obtained from the sample after the incubation, wherein the difference indicates the first competitor peptide is an MHC-binding peptide for the monomer.

2. (Currently Amended) The method of claim 1, wherein the excess amount of the first competitor peptide is about or more than 100-fold molar excess.

3. (Original) The method of claim 1, wherein the tracer peptide displaces at least 90% of the template peptide in a parallel competition assay conducted in the absence of the first competitor peptide.

4. (Currently Amended) The method of claim 1, wherein ~~the suitable~~ said liquid phase ~~conditions include~~ condition includes incubating the sample for about 2 to 20 hours.

5. (Currently Amended) The method of claim 4, wherein ~~the suitable~~ said liquid phase ~~conditions~~ condition further ~~include~~ includes incubating the sample at about 21 °C.

6. (Original) The method of claim 1, wherein the detectable label is a fluorophore.

7. (Withdrawn) The method of claim 6, wherein the monomer is attached to a solid support prior to determining the signal produced solely by the monomers in the sample.

8. (Withdrawn) The method of claim 7, wherein the MHC monomer or modified MHC monomer is biotinylated and the monomer is attached to the solid support via a biotin/avidin or streptavidin linkage.

9. (Original) The method of claim 6, wherein the fluorophore is fluorescein (FITC).

10. (Cancelled)

11. (Original) The method of claim 1, wherein the difference is a decrease in the signal and binding of the first competitor peptide to the monomer is proportional to the amount of the decrease.

12. (Cancelled)

13. (Currently Amended) The method of claim [[12]] 1, wherein the monomer is HLA-A*020/Mart-1 26-35.

14. (Original) The method of claim 13, wherein the tracer peptide is HBc 18-27.

15. (Withdrawn-Amended) The method of claim [[12]] 1, wherein the monomer is obtained from the sample in b) by cytometry.

16. (Withdrawn) The method of claim 1, wherein the method is repeated, except that a different competitor peptide is used.

17 - 19. (Canceled)

20. (Previously Presented) The method of claim 1, wherein the first competitor peptide comprises from about 8 to about 12 amino acids.

21. (Original) The method of claim 20, wherein the monomer remains folded during the assay.

22. (Original) The method of claim 20, wherein the tracer peptide comprises from about 8 to about 12 amino acids and peptide exchange occurs without unfolding or denaturing of the monomer.

23. (Withdrawn) The method of claim 1, wherein the affinity of an exchanged competitor peptide is substantially equal to affinity of the first competitor peptide when folded into the binding pocket of the monomer during reconstitution of a ternary complex comprising the first competitor peptide and the monomer.

24. (Withdrawn) The method of claim 1, wherein the modified MHC monomer comprises cell surface domains of the MHC monomer but does not comprise other domains of the MHC monomer.

25. (Original) The method of claim 1, wherein the allele of the monomer is known and the determining indicates whether the first competitor peptide is specific for the allele of the monomer.

26. (Currently amended) A method for measuring relative affinity of MHC-binding peptides for an MHC monomer, or modified MHC monomer, said method comprising:

a) incubating under a suitable liquid phase conditions condition a sample a ternary complex comprising:

at least one ~~MHC~~ HLA-A2 monomer or modified ~~MHC~~ HLA-A2 monomer having bound thereto a template MHC-binding peptide and, wherein the MHC monomer is HLA-A2, further comprising beta-2 microglobulin, and wherein the modified MHC HLA-A2 monomer maintains the ability to assemble into a ternary complex with the template MHC-binding peptide and beta-2 microglobulin,[[,]] and wherein said monomer is produced in an expression system selected from the group consisting of a prokaryotic system, a yeast system, a plant system, and an insect system,

an excess amount of a first competitor peptide, and

a tracer MHC-binding peptide tagged with a detectable label, wherein said tracer peptide competes with the first competitor peptide and the template peptide for binding to the monomer,

~~so as to allow competition between the first competitor peptide, the template peptide, and the tracer peptide for binding to the MHC monomer or modified MHC monomer,~~
wherein the template peptide has lower affinity than the tracer peptide for the monomer; and
wherein at least a portion of the first competitor peptide exchanges with the template peptide;
and

b) determining a difference in signal produced by the detectable label in the total sample as compared with signal produced solely by monomer obtained from the sample after the incubation, wherein the difference indicates affinity of the first competitor peptide for the monomer.

27. (Currently Amended) The method of claim 26, wherein the excess amount of the first competitor peptide is about or more than 100-fold molar excess.

28. (Original) The method of claim 26, wherein the tracer peptide displaces at least 90% of the template peptide in a competition peptide exchange assay conducted in the absence of the first competitor peptide.

29. (Currently Amended) The method of claim 26, wherein ~~the suitable~~ said liquid phase conditions include condition includes incubating the sample for about 2 to about 6 hours.

30. (Currently Amended) The method of claim 26, wherein ~~the suitable~~ said liquid phase conditions condition further ~~include~~ includes incubating the sample at about 21 °C.

31. (Original) The method of claim 26, wherein the detectable label is a fluorophore.

32. (Withdrawn) The method of claim 31, wherein the monomer is attached to a solid support prior to determining the signal produced solely by the monomers in the sample.

33. (Withdrawn-Amended) The method of claim 32, wherein the ~~MHC~~ HLA-A2 monomer or modified ~~MHC~~ HLA-A2 monomer is biotinylated and the monomer is attached to the solid support via a biotin/avidin or streptavidin linkage.

34. (Original) The method of claim 31, wherein the fluorophore is fluorescein (FITC).

35. (Cancelled)

36. (Original) The method of claim 26, wherein the difference is a decrease in the signal and binding of the first competitor peptide to the monomer is proportional to the amount of the decrease.

37. (Cancelled)

38. (Currently Amended) The method of claim ~~[[37]]~~ 26, wherein the monomer is HLA-A*020/Mart-1 27-35.

39. (Original) The method of claim 26, further comprising obtaining the monomer from the sample in b) by cytometry.

40. (Withdrawn) The method of claim 26, wherein the method is repeated, except that a different competitor peptide is used.

41. (Withdrawn) The method of claim 40, wherein the tracer peptide is HBc 18-27.

42 - 44. (Canceled)

45. (Previously Presented) The method of claim 26, wherein the first competitor peptide comprises from about 8 to about 12 amino acids.

46. (Original) The method of claim 45, wherein peptide exchange occurs without unfolding or denaturing of the monomer.

47. (Original) The method of claim 45, wherein the tracer peptide comprises from about 8 to about 12 amino acids and peptide exchange occurs without unfolding or denaturing of the monomer.

48. (Withdrawn) The method of claim 26, wherein the affinity of a exchanged competitor peptide is substantially the equal to affinity of the first competitor peptide when folded into the binding pocket of the monomer during reconstitution of a ternary complex comprising the first competitor peptide and the monomer.

49. (Withdrawn-Amended) The method of claim 26, wherein the modified MHC HLA-A2 monomer comprises cell surface domains of the ~~MHC~~ HLA-A2 monomer but does not comprise other domains of the ~~MHC~~ HLA-A2 monomer.

50. (Original) The method of claim 26, wherein the allele of the monomers is known and the determining determines whether the first competitor peptide is specific for the allele of the monomer.

51-72. (Canceled)

73. (Currently amended) A ~~system~~ kit for identifying an MHC-binding peptide for an MHC monomer, or modified MHC monomer, said ~~system~~ kit comprising:

a) a ternary complex comprising at least one ~~MHC~~ HLA-A2 monomer or modified ~~MHC~~ HLA-A2 monomer having bound thereto a template MHC-binding peptide and, wherein the MHC monomer is HLA-A2, further comprising beta-2 microglobulin, and wherein the modified ~~MHC~~ HLA-A2 monomer maintains the ability to assemble into a ternary complex with the template MHC-binding peptide and beta-2 microglobulin, and wherein said monomer is produced in an expression system selected from the group consisting of a prokaryotic system, a yeast system, a plant system, and an insect system, and

b) a tracer MHC-binding peptide tagged with a detectable label wherein the template peptide has lower affinity than the tracer peptide for the monomer.

74. (Currently Amended) The ~~system~~ kit of claim 73, wherein the system further comprises an instruction for using the system.

75. (Currently Amended) The ~~system~~ kit of claim 73, wherein the monomer is HLA-A*020 and the template peptide is Mart-1 26-35.

76. (Currently Amended) The ~~system~~ kit of claim 73, wherein the tracer peptide is HBc 18-27.

77. (Currently Amended) The ~~system~~ kit of claim 73, wherein the detectable label is FITC.

78. (Withdrawn-Amended) The ~~system~~ kit of claim 73, wherein the monomer is biotinylated for attachment to an avidinated solid support.

79. (New) The method of claim 1, wherein said expression system is a prokaryotic system.

80. (New) The method of claim 79, wherein said prokaryotic system is in *E.coli*.

81. (New) The method of claim 26, wherein said expression system is a prokaryotic system.

82. (New) The method of claim 81, wherein said prokaryotic system is in *E.coli*.

83. (New) The kit of claim 73, wherein said expression system is a prokaryotic system.

84. (New) The kit of claim 83, wherein said prokaryotic system is in *E.coli*.